FIG. 1-1

Constitutively Active Receptors

File Name	Receptor	Mutation Site	Sequence	Assay / Cells	Reference
CLASS A GROUP I					
MSHR_mouse	melanocyte-stimulating hormone MSH	TMII	92 VSIVLETTIIL SEQ ID NO: 2 K	adenylyl cyclase activity/ HEK293, *stably transfected*	(Robbins, Nadeau et al. 1993)
CLASS A GROUP II SH1B_human	5-hydroxytryptamine$_{1B}$	C-terminus of IC3	313 RERKATKTLGI SEQ ID NO: 3 K,R,Q	binding of [^{35}S]GTP[S] / CHO-KI	(Pauwels, Gouble et al. 1999)
5H2A_human	5-hydroxytryptamine$_{2A}$	C-terminus of IC3	322 NEQKACKVLGI SEQ ID NO: 4 K	IP production / COS-7	(Egan, Herrick-Davis et al. 1998)
2H2C_rat	5-hydroxytryptamine$_{2C}$	C-terminus of IC3	312 NEDDASKVLGI SEQ ID NO: 5 L	PI hydrolysis / COS-7	(Herrick-Davis, Egan et al. 1997)

FIG. 1-2

CLASS A GROUP II					
A1AB_human	α_{1B}-adrenergic alpha 1B-AR	TMDI junction between TMDIII and IC2	63 FAIVGNILVIL SEQ ID NO: 6 A 142 CAISIDRYIGV SEQ ID NO: 7 A	IP / COS-7	(Scheer, Fanelli et al. 1997)
A1AB_human	α_{1B}-adrenergic alpha 1B-AR	junction between TMDIII and IC2	143 CAISIDRYIGV SEQ ID NO: 8 K	IP / COS-7	(Scheer, Costa et al. 2000)
A1AB_human	α_{1B}-adrenergic	TMIII carboxyl end of IC3 TMV	128 AVDVLCCTASI SEQ ID NO: 9 F 293 REKKAAKTLGI SEQ ID NO: 10 E 204 EEPFYALFSSLG SEQ ID NO: 11 V	IP / COS-1 IP arachidonic acid release IP / COS-1	(Perez, Hwa et al. 1996) (Hwa, Gaivin et al. 1997)
A1AB_human	α_{1B}-adrenergic	C-terminal IC3	293 SREKKAAKT SEQ ID NO: 12 X=19 different substitutions	PI / COS-7	(Kjelsberg, Cotecchia et al. 1992)
A1AB_human	α_{1B}-adrenergic	C-terminus IC3	288 293 KFSREKKAAKTLGI SEQ ID NO: 13 K H L	PI hydrolysis / rat fibroblast	(Allen, Lefkowitz et al. 1991)
A2AA_human	α_2C10-adrenergic alpha-2AAR	C-terminal IC3 loop	373 (348?) EKRFTFVLAV SEQ ID NO: 14 X=F,A,C,E,K	adenylyl cyclase inhibition / HEK293	(Ren, Kurose et al. 1993)
ACM1_human	muscarinic Hm1 muscarinic acetylcholine M1	C-terminal IC3 loop junction	360 SLVKEKKAARTLS SEQ ID NO: 15 A	PI / HEK(U293)	(Högger, Shockley et al. 1995)
ACM2-human	muscarinic acetylcholine M2	junction of IC3 and TMVI	390 KKVTRTIL↑A SEQ ID NO: 16 1-4 A inserted	IP production, inhibition of cAMP production / COS-7	(Liu, Blin et al. 1996)

FIG. 1-3

CLASS A GROUP II					
ACM3_rat	m3 muscarinic (rat) muscarinic acetylcholine M3	TMVI	507 TWTPYNIMVLVNT SEQ ID NO: 17 S	IP / COS-7	(Blüml, Mutschler et al. 1994)
ACM5_human	m5 muscarinic muscarinic acetylcholine M5	N-terminus to TMII TMVI	chimera composed of m2 1-69 m5 77-445 m2 391-466	β-gal / NIH 3T3	(Burstein, Spalding et al. 1996)
ACM5_human	m5 muscarinic muscarinic acetylcholine M5	TMVI SEQ ID NO: 18	451 459 465 AILLA EIITW TPYNI MVLVST M L H C V S F T	β-gal; radioligand binding / NIH-3T3	(Spalding, Burstein et al. 1998)
ACM5_human	m5 muscarinic muscarinic acetylcholine M5	junction of TMVI and EC3	465 YNIMVLVSTFCDKCV SEQ ID NO: 19 X=V,F,R,K,+more	β-gal; radioligand binding / NIH-3T3	(Spalding, Burstein et al. 1997)
B1AR_human	β_1-adrenergic	C-terminus	389 RKAFQGLLCCA SEQ ID NO: 20 R	adenylyl cyclase; agonist binding / CHW	(Mason, Moore et al. 1999)
B2AR_human	β_2-adrenergic beta-2AR	C-terminal IC3 loop	266 272 FCLKEHKALKTLGI SEQ ID NO: 21 SR K A	adenylyl cyclase activation; agonist binding affinity / COS-7 or CHO	(Samama, Cotecchia et al. 1993); (Lefkowitz, Cotecchia et al. 1993)
DADR_human	dopamine D1A	carboxyl terminal IC3	264 SFKMSFKRETKVLKT SEQ ID NO: 22 I K 288 *from D1B receptor* APDTSIKKETKVLKT SEQ ID NO: 23	adenylyl cyclase; cAMP accumulation / HEK293	(Charpentier, Jarvie et al. 1996)
DADR_human	dopamine D1	TMVI	286 FVCCWLPFFIL SEQ ID NO: 24 A	cAMP accumulation / COS-7	(Cho, Taylor et al. 1996)
HH2R_rat	histamine H_2	IC2	115 FMISLDRYCAV SEQ ID NO: 25 N,A	cAMP production / HEK-293	(Alewijnse, Timmerman et al. 2000)

FIG. 1-4

File Name	Receptor	Mutation Site	Sequence	Assay / Cells	Reference
CLASS A GROUP III					
OPSD_human	opsin rhodopsin	TMII TMIII TMVII	90 FMVLGGFTSTLY SEQ ID NO: 26 D 113 GCNLEGFFAT SEQ ID NO: 27 Q 292 296 MTIPAFFAKSAAIY SEQ ID NO: 28 E G,E,M ^{292}Ala neutral a.a converted to carboxylate and competes with ^{113}Glu for salt bridge with ^{296}Lys	transducin; phosphorylation by rhodopsin kinase / COS	(Rim and Oprian 1995)
OPSD_human	opsin rhodopsin	TMIII	134 VVLAIERYVVV SEQ ID NO: 29 I,Q,S	transducin; radioligand binding / COS	(Acharya and Karnik 1996)
OPSD_human	opsin rhodopsin	TM6 *plus* TM3	257 RMVIIMVIAFL SEQ ID NO: 30 Y,N *plus* G113Q	transducin, GTPγS uptake / COS	(Han, Smith et al. 1998)
OPSD_human	opsin rhodopsin	TMVII IC2	296 PAFFAKSAAIY SEQ ID NO: 31 G X=E,M natural mutants + 10 different a.a. substitutions disrupts critical salt bridge between ^{296}Lys(TMVII) and ^{113}Glu(TMIII) 134 VVLAIERYVVV SEQ ID NO: 32 Q	transducin; radioligand binding / COS	(Govardhan and Oprian 1994); (Cohen, Yang et al. 1993) (Cohen, Yang et al. 1993)

FIG. 1-5

TRFR_mouse	thyrotropin-releasing hormone TRH-R	carboxyl tail	335 FRKLCNCKQK STOP SEQ ID NO: 33	$^{45}Ca^{2+}$ efflux, $[Ca^{2+}]$ / Xenopus oocytes; IP formation / AtT20, *stably transfected*	(Matus-Leibovitch, Nussenzveig et al. 1995)

FIG. 1-6

File Name	Receptor	Mutation Site	Sequence	Assay / Cells	Reference
CLASS A GROUP IV					
BRB2_human	bradykinin B_2 B2 bradykinin BK-2	TMIII TMVI	113 AIISMNLYSSI SEQ ID NO: 34 A 256 LLFIICWLPFQI SEQ ID NO: 35 F	IP production / COS-7	(Marie, Koch et al. 1999)

FIG. 1-7

File Name	Receptor	Mutation Site	Sequence	Assay / Cells	Reference
CLASS A GROUP V					
AG2R_rat	AT_{1A} Type-1A angiotensis II	TMIII	111 ASVSFNLYASV SEQ ID NO: 36 A disrupts ^{111}Asn(TMIII)-^{292}Tyr(TMVII) interaction	phospholipase C; IP production / COS-7	(Groblewski, Maigret et al. 1997)
AG2R_rat	AT_{1A} Type-1A angiotensis II	C-terminus of TM7 other multiple mutations	305 LFYGFLGKKFK SEQ ID NO: 37 Q	IP production / HEK-293; intrcellular Ca^{2+} mobilization / CHO	(Parnot, Bardin et al. 2000)
FMLR_human	formylmethionylleucylphenylalanine (fMLPR)	IC1 SEQ ID NO: 38 SEQ ID NO: 39	51 LVIWVAGFRMTHTVTTISYLNKAVA LVVWVTAFEAKRTINAIWFLNLAVA (K above conflicts with SWISS-PROT database)	PI production; phospholipase C stimulation / COS-7	(Amatruda, Dragas-Graonic et al. 1995)
IL8B_human	interleukin-8 receptor B CXCR-2 chemokine	IC2	138 ACISVDRYLAIVH SEQ ID NO: 40 V	IP production; Ca^{2+} moblization and actin polymerization / NIH 3T3	(Burger, Burger et al. 1999)
LSHR_human	luteinizing hormone (LH)	IC3	564 MATNKDTKIAKK SEQ ID NO: 41 G	cAMP production / HEK293	(Kudo, Osuga et al. 1996)
LSHR_human	luteinizing hormone (LH)	TMVI	578 ILIFTDFTCMA SEQ ID NO: 42 G	cAMP production / COS-7	(Shenker, Laue et al. 1993)
LSHR_human	luteinizing hormone (LH)	TM6 SEQ ID NO: 43	571 577 KIAKKMAILIFTDFTCM I I	cAMP production / COS-7	(Kosugi, Van Dop et al. 1995)
LSHR_rat	luteinizing hormone / human chorionic gonadotropin (LH/hCG)	TMVI	556 ILIFTDFTCMA SEQ ID NO: 44 G, Y	cAMP production / HEK 293T	(Bradbury, Kawate et al. 1997; Bradbury and Menon 1999)
OPRD_mouse	delta opiod receptor	TM3	128 KVLSIDYYNMF SEQ ID NO: 45 A, K, H	adenylyl cyclase inhibition / COS-7	(Cavalli, Babey et al. 1999)
OXYR_human	oxytocin	IC2	137 LMSLDRCLAIC SEQ ID NO: 46 A	IP production / COS-7	(Fanelli, Barbier et al. 1999)

FIG. 1-8

PAFR_human	platelet-activating factor (PAF)	C-terminus of IC3	231 EVKRRALWMVCTVLAV SEQ ID NO: 47 R	IP production / COS-7	(Parent, Le Gouill et al. 1996)
PAFR_human	platelet-activating factor (PAF)	TMIII	100 CLFFINTYCSV SEQ ID NO: 48 A	arachnidonate release, IP production, adenylyl cylcase inhibition / CHO	(Ishii, Izumi et al. 1997)
PE23_human	prostaglandin E_3, EP3III EP3IV	C-terminal tail	360 FCQEEFWGN SEQ ID NO: 49 FCQMRKRRLREQEEFWGN SEQ ID NO: 50 ↑truncated	inhibition of adenylyl cyclase / CHO-K1	(Jin, Mao et al. 1997)
PE23_mouse	prostaglandin E_3 EP3	carboxyl-terminal tail SEQ ID NO: 51	336 KILLRKFCQIRDHT (3α) MMNHL (3β) ↑truncated	inhibition of adenylate cyclase / CHO, *stably expressed*	(Hasegawa, Negishi et al. 1996)
THRR_human	thrombin	EC2 loop SEQ ID NO: 52	259 268 CHDVLNETLLEGYYAYY DLKD KDF I	$^{45}Ca^{2+}$ efflux, PI hydrolysis, reporter gene induction / COS-7	(Nanevicz, Wang et al. 1996)
TSHR_human	thyrotropin (TSHR) thyroid stimulating hormone	EC1 EC2	486 YYNHAIDWQTG SEQ ID NO: 53 F,M 568 YAKVSICLPMD SEQ ID NO: 54 T	inositol phosphate--diacylglycerol cascade / COS-7	(Parma, Van Sande et al. 1995)
TSHR_human	thyrotropin (TSHR) thyroid stimulating hormone	TMIII TMVII	509 ASELSVYTLTV SEQ ID NO: 55 A 672 YPLNSCANPFL SEQ ID NO: 56 Y	adenylyl cyclase activation / COS-7	(Duprez, Parma et al. 1994)
TSHR_human	thyrotropin (TSHR) thyroid stimulating hormone	TMV	597 VAFVIVCCCHV SEQ ID NO: 57 L	cAMP formation / COS-7 cells	(Esapa, Duprez et al. 1999)
TSHR_human	thyrotropin (TSHR) thyroid stimulating hormone	TMVII	677 CANPFLYAIFT SEQ ID NO: 58 V	cAMP formation / CHO cells	(Russo, Wong et al. 1999)
TSHR_human	thyrotropin (TSHR) thyroid stimulating hormone	IC3	613 621 VRNPQYNPGDKDTKIAK deletion SEQ ID NO: 59	cAMP formation / COS-7	(Wonerow, Schoneberg et al. 1998)

FIG. 1-9

TSHR_human	thyrotropin (TSHR) thyroid stimulating hormone	IC3 / TMVI SEQ ID NO: 60	623 632 KDTKIAKRMAVLIFTDFICM V I	cAMP activation / COS-7	(Paschke, Tonacchera et al. 1994)
V2R_human	vasopressin V2	IC2 SEQ ID NO: 61	136 LAMTLDRHRAI A	cAMP formation / COS-7	(Morin, Cotte et al. 1998)

FIG. 1-10

File Name	Receptor	Mutation Site	Sequence	Assay / Cells	Reference
CLASS B GROUP I					
CALR_human	human calcitonin hCTR-1 hCTR-2	wild type (native) protein		adenylyl cyclase cAMP production / COS-1	(Cohen, Thaw et al. 1997)
CLASS B GROUP II					
PTRR_human	parathyroid hormone PTH / PTH-related peptide	junction of IC1 and TMII junction of IC3 and TMVI	223 TRNYIHMHLFL SEQ ID NO: 62 R,K 410 KLLKSTLVLMP SEQ ID NO: 63 C,others	cAMP accumulation / COS-7	(Schipani, Jensen et al. 1997)
CLASS B GROUP III					
GIPR_human	glucose-dependent insulinotropic peptide (GIP-R)	TMVI	340 VFAPVTEEQAR SEQ ID NO: 64 P	cAMP production / L293	(Tseng and Lin 1997)
GLR_rat	glucagon	junction of IC loop1 and TMII IC end of TMVI	178 TRNYIHGNLFA SEQ ID NO: 65 R 352 RLARSTLTLIP SEQ ID NO: 66 A	cAMP accumulation / COS-7	(Hjorth, Orskov et al. 1998)
VIPR_human	vasoactive intestinal peptide 1 (VIP)	junction of IC loop 1 and TMII junction of IC loop 3 and TMVI	178 RNYIHMHLFI SEQ ID NO: 67 R *requires functional integrity of the N-terminal EC domain* 343 LARSTLLLIP SEQ ID NO: 68 X= K,P	cAMP production / COS-7 or CHO	(Gaudin, Maoret et al. 1998) (Gaudin, Rouyer-Fessard et al. 1998)

FIG. 1-11

File Name	Receptor	Mutation Site	Sequence	Assay / Cells	Reference
CLASS C					
CASR_human	calcium-sensing	N-terminal EC	TLSFVAQNKIDSLNLDEFCNCSEHI *various substitutions, in multiple combinations* SEQ ID NO: 69	IP / tsA	(Jensen, Spalding et al. 2000)

FIG. 1-12

File Name	Receptor	Mutation Site	Sequence	Assay / Cells	Reference
CLASS D					
O74283 RCB2 C. cinereus	pheromone	TM6	229 PLSAYQIYLGT SEQ ID NO: 70 P	heterologous yeast assay	(Olesnicky, Brown et al. 1999)
STE2_yeast	pheromone α-factor	TM6	258 QSLLVPSIIFI SEQ ID NO: 71 LL	*lacZ* reporter gene	(Konopka, Margarit et al. 1996)
STE2_yeast	pheromone α-factor	double mutations TM5 and TM6	223 MSFVLVVKLILAIR SEQ ID NO: 72 C C 247 251 DSFHILLIMSCQSLL SEQ ID NO: 73 CC CC *double mutations* shaded *double mutations*	*lacZ* reporter gene / yeast	(Dube, DeCostanzo et al. 2000)
STE3_yeast	pheromone a-factor	IC3	194 DVRDILHCTNS SEQ ID NO: 74 Q	β-galactosidase	(Boone, Davis et al. 1993)
STE2_yeast	pheromone α-factor	TM6	253 258 LIMSCQSLLVPSIIFI SEQ ID NO: 75 L LP	β-galactosidase	(Sommers, Martin et al. 2000)

FIG. 1-13

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FIG. 2

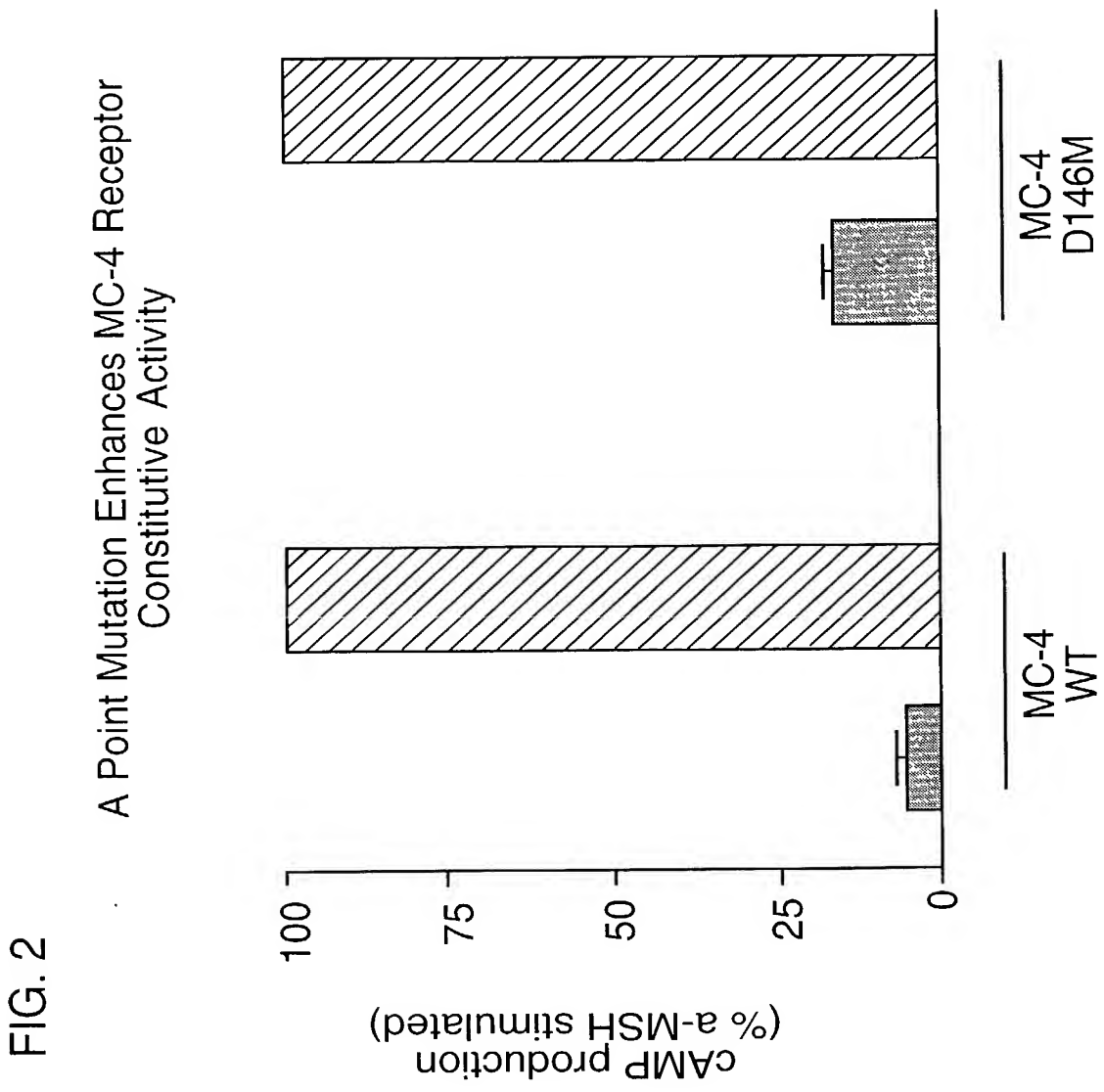

A Point Mutation Enhances MC-4 Receptor Constitutive Activity
basal
α-MSH 1 μM
cAMP production (% a-MSH stimulated)
100
75
50
25
0
MC-4 WT
MC-4 D146M

FIG. 3

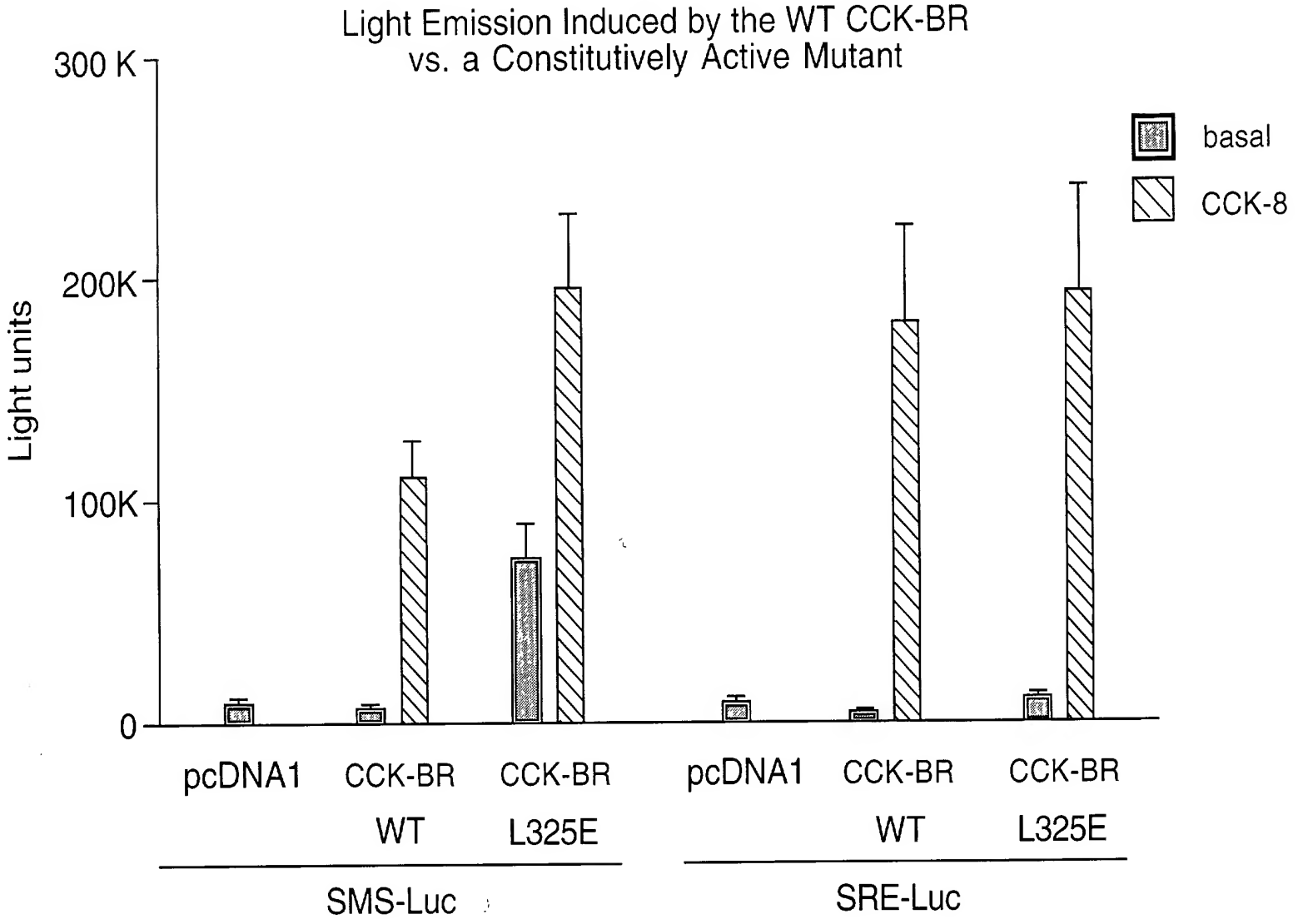

Light Emission Induced by the WT CCK-BR
vs. a Constitutively Active Mutant
300 K
200K
100K
0
Light units
basal
CCK-8
pcDNA1
CCK-BR
WT
CCK-BR
L325E
pcDNA1
CCK-BR
WT
CCK-BR
L325E
SMS-Luc
SRE-Luc

FIG. 4

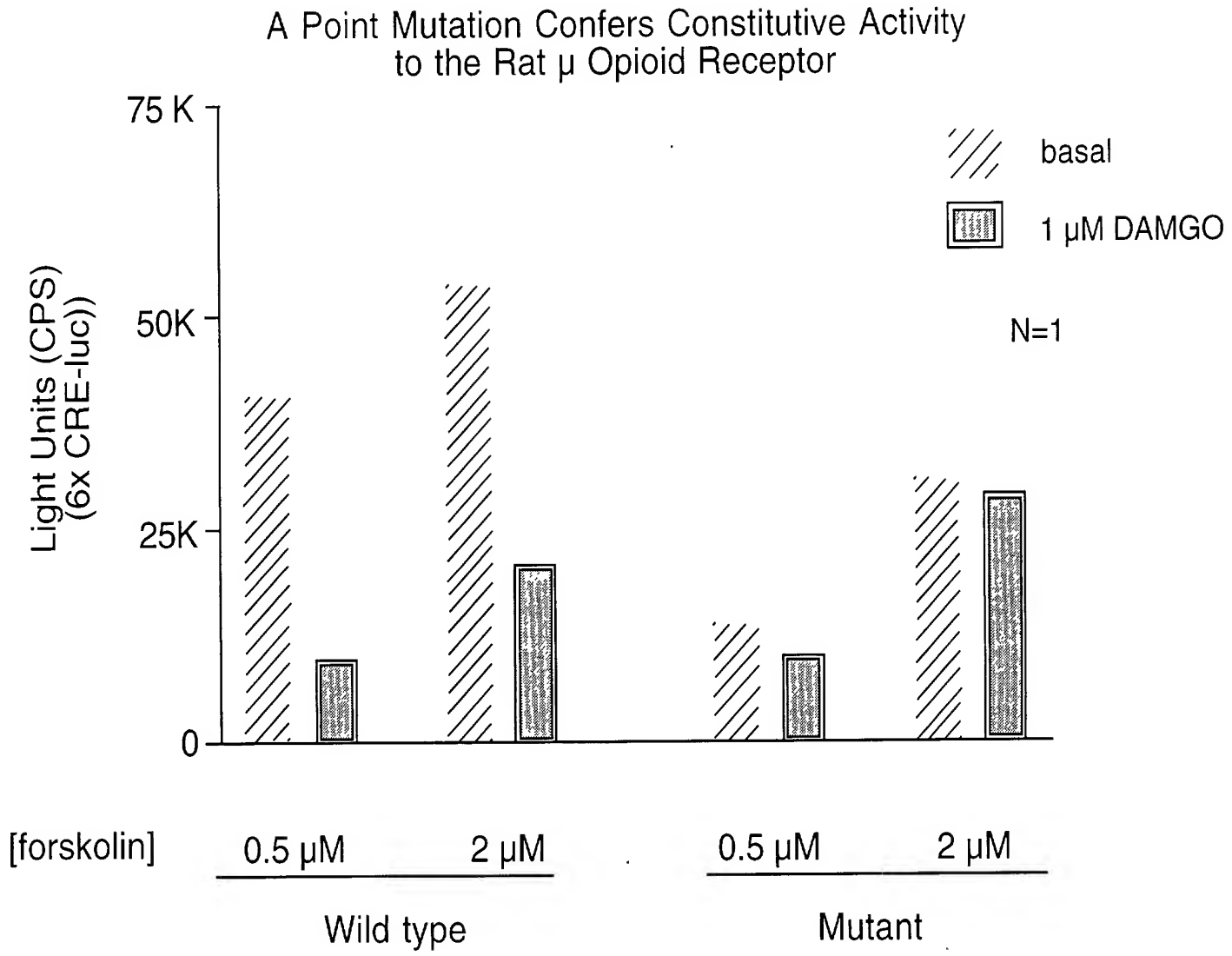

A Point Mutation Confers Constitutive Activity
to the Rat μ Opioid Receptor
75 K
50K
25K
0
Light Units (CPS)
(6x CRE-luc))
basal
1 μM DAMGO
N=1
[forskolin]
0.5 μM
2 μM
0.5 μM
2 μM
Wild type
Mutant

FIG. 5

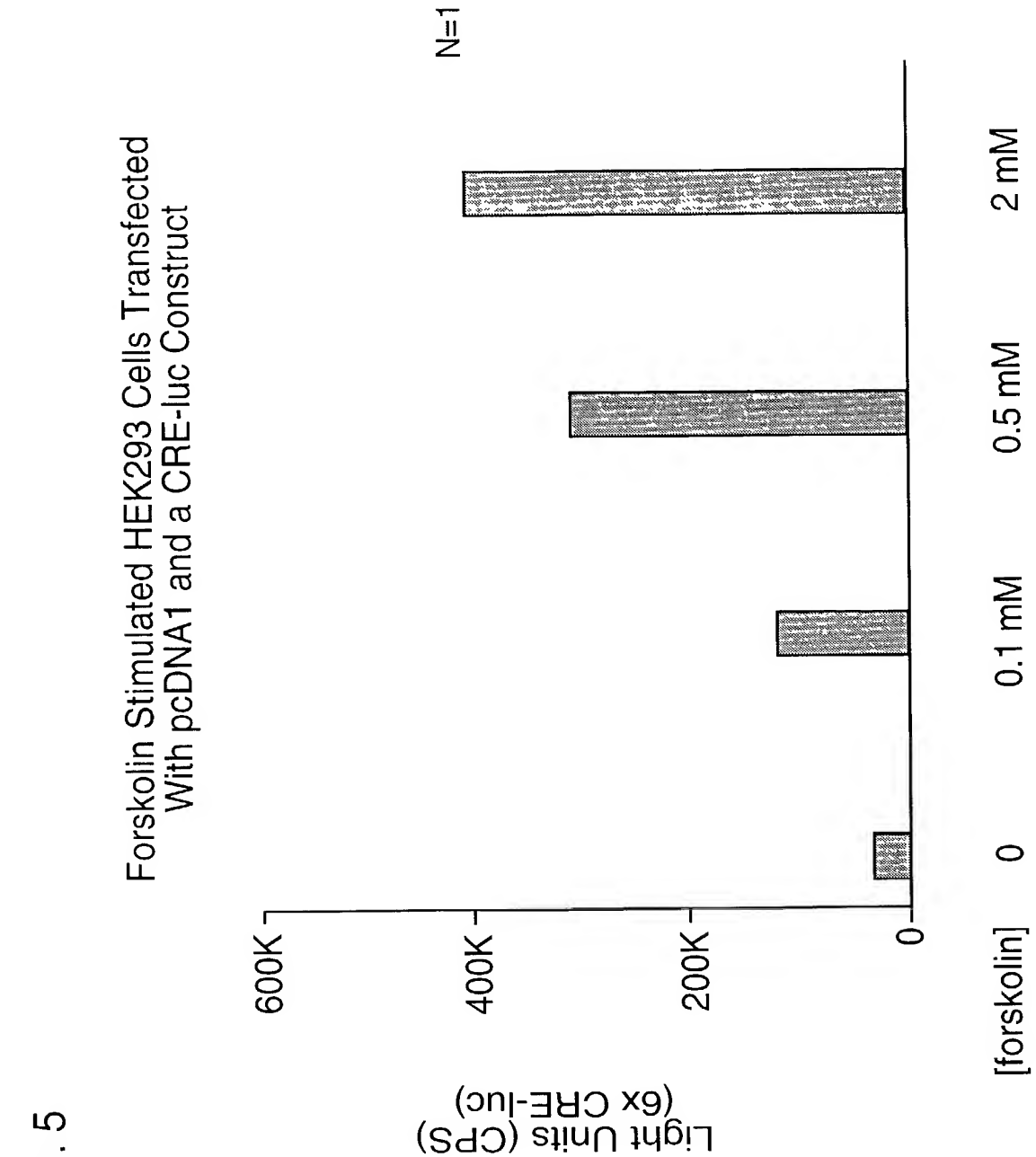

Forskolin Stimulated HEK293 Cells Transfected
With pcDNA1 and a CRE-luc Construct
N=1
Light Units (CPS)
(6x CRE-luc)
600K
400K
200K
0
[forskolin]
0
0.1 mM
0.5 mM
2 mM

FIG. 6

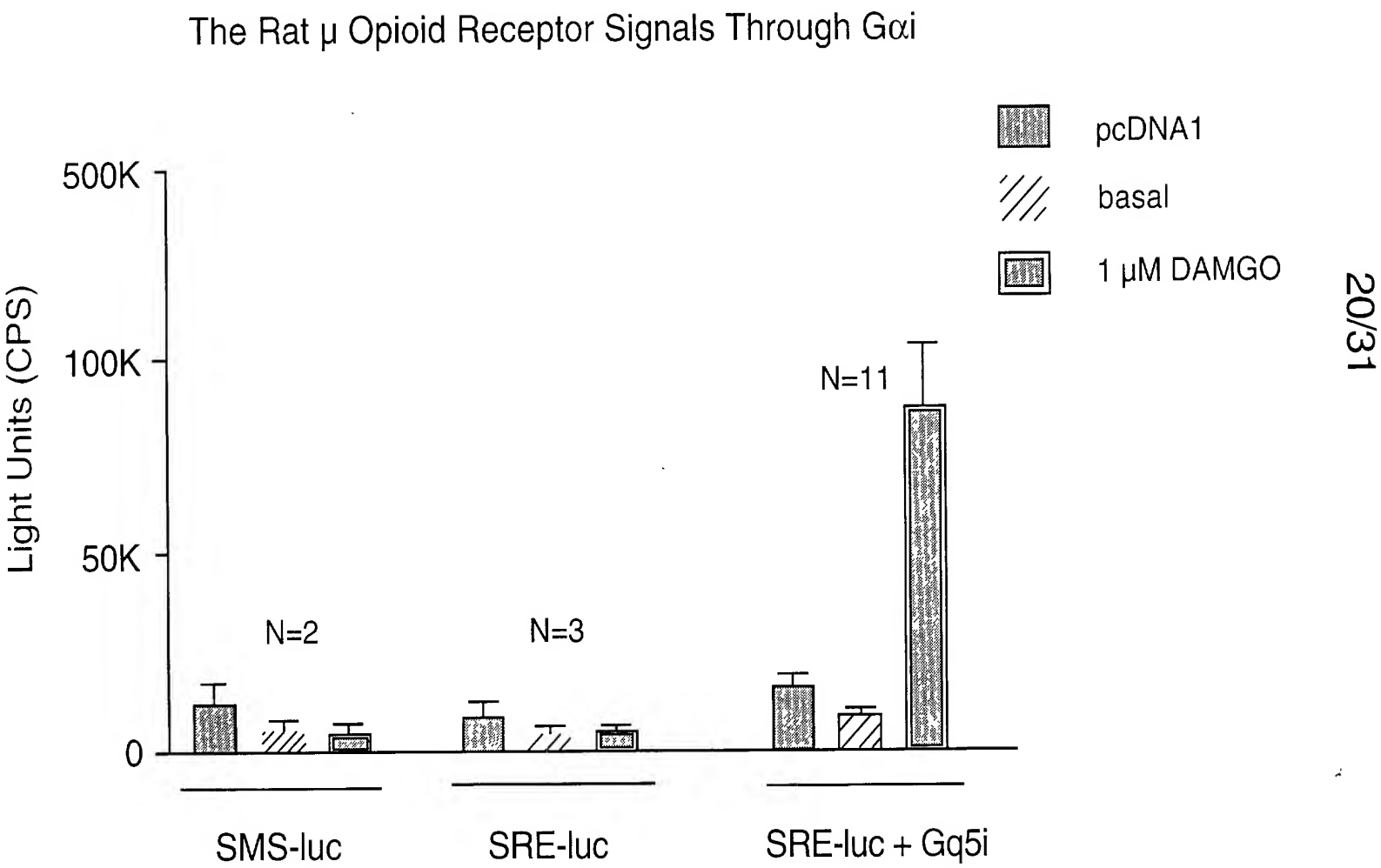

The Rat μ Opioid Receptor Signals Through Gαi
pcDNA1
basal
1 μM DAMGO
Light Units (CPS)
500K
100K
50K
0
N=2
N=3
N=11
SMS-luc
SRE-luc
SRE-luc + Gq5i

FIG. 7

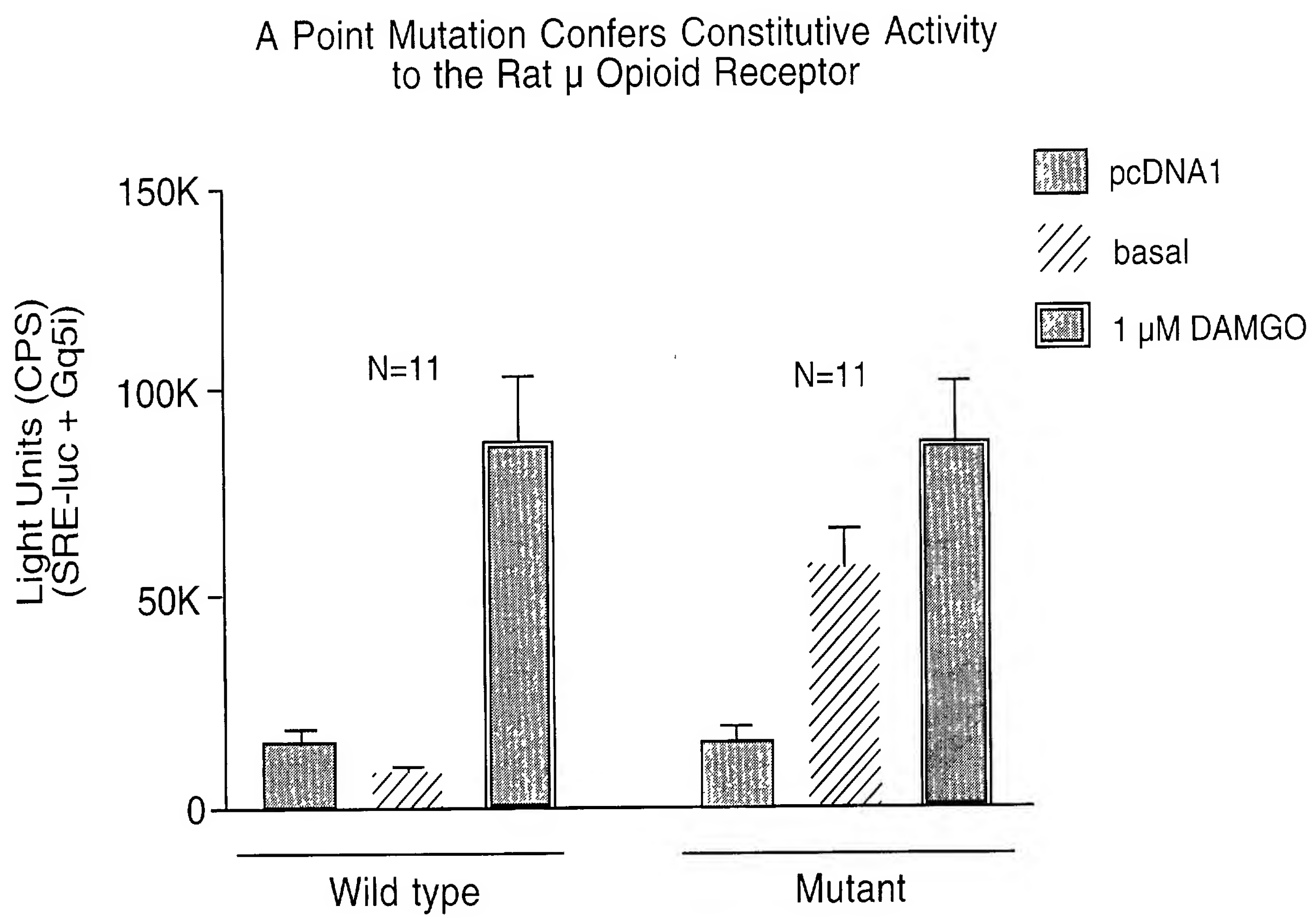


FIG. 8

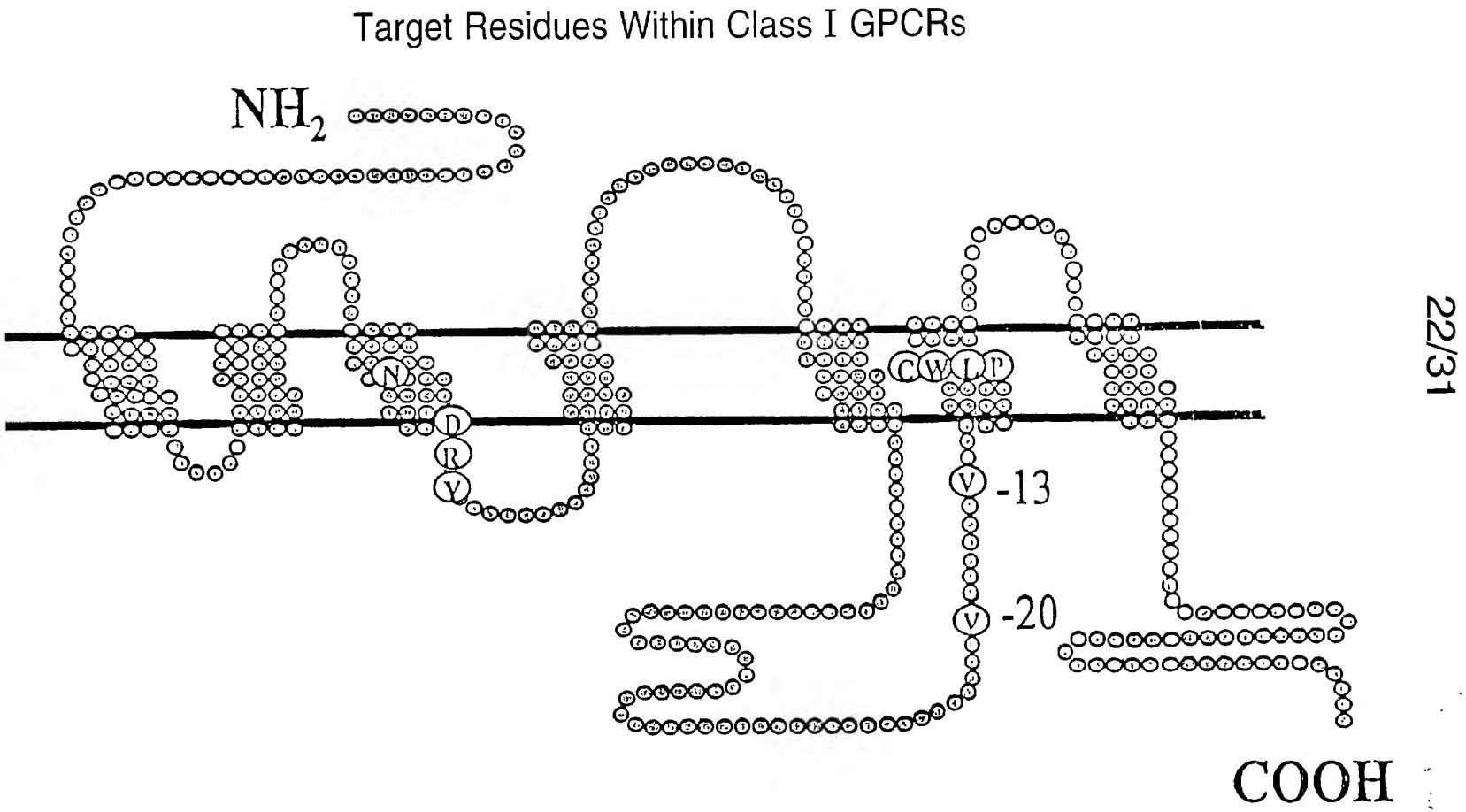

Target Residues Within Class I GPCRs
NH2
N
D
R
Y
C
W
L
P
V
-13
V
-20
COOH

FIG. 9

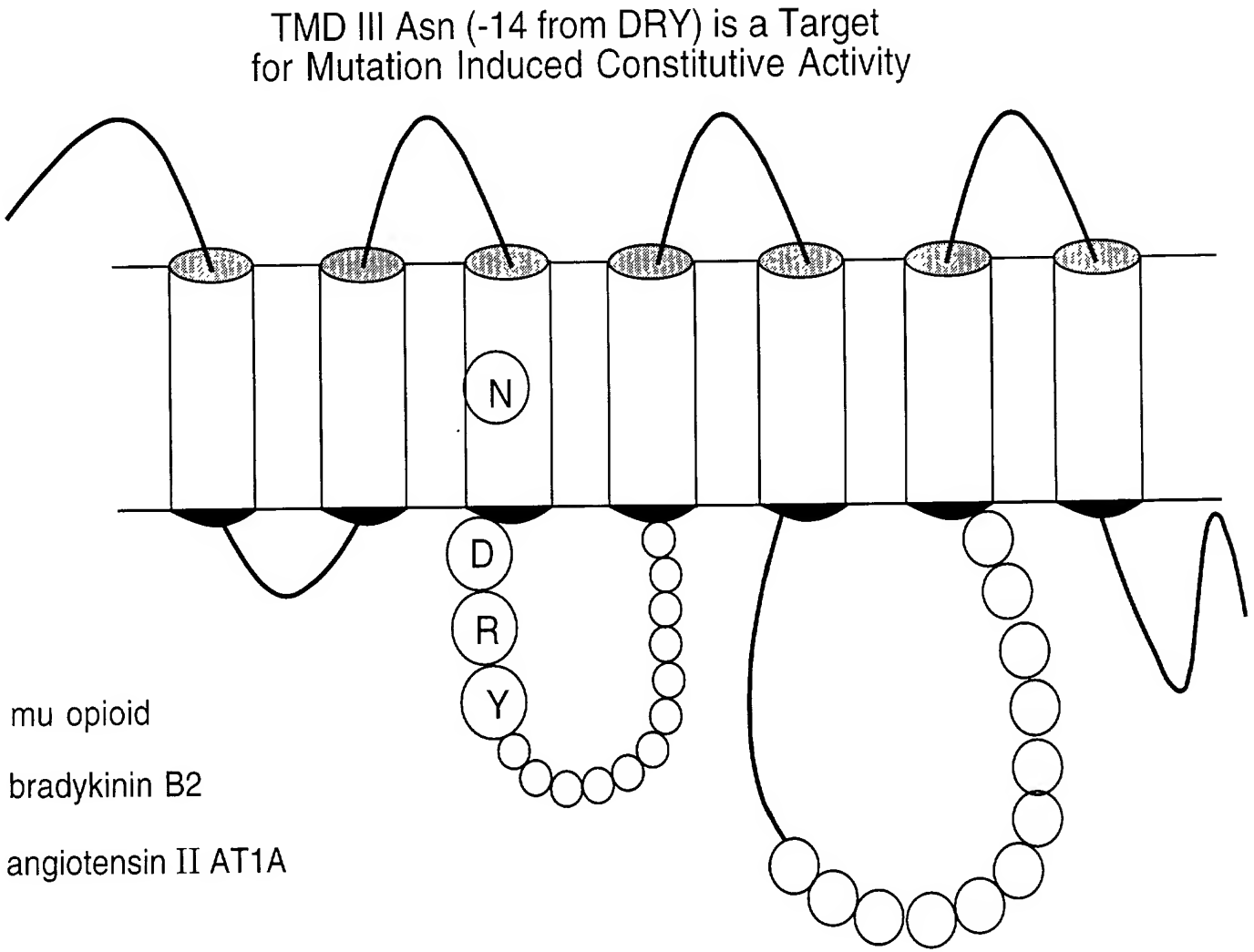
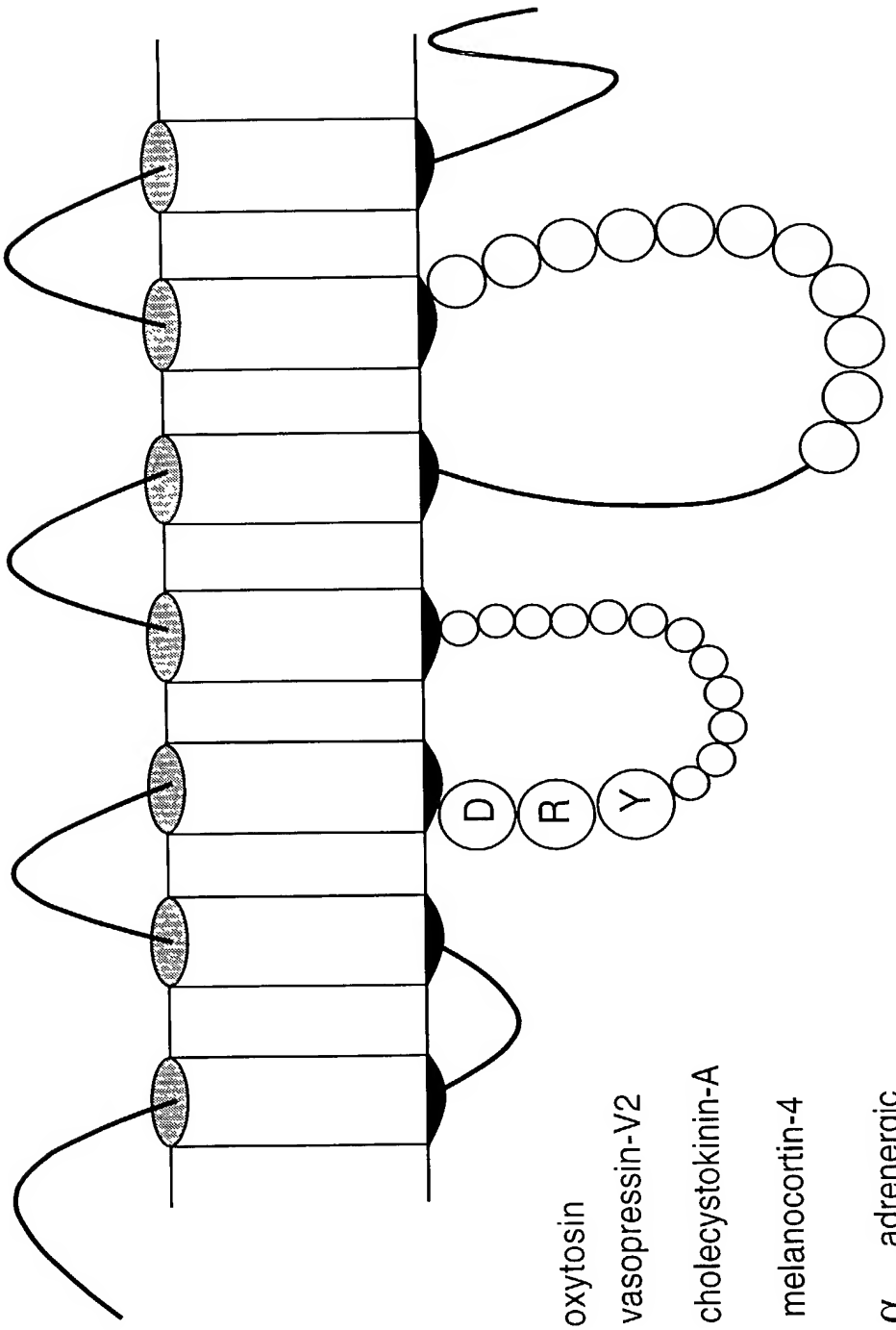


FIG. 10

The 'DRY' Motif is a Target for Mutation Induced Constitutive Activity



oxytosin

vasopressin-V2

cholecystokinin-A

melanocortin-4

α_{1B} adrenergic

FIG. 11

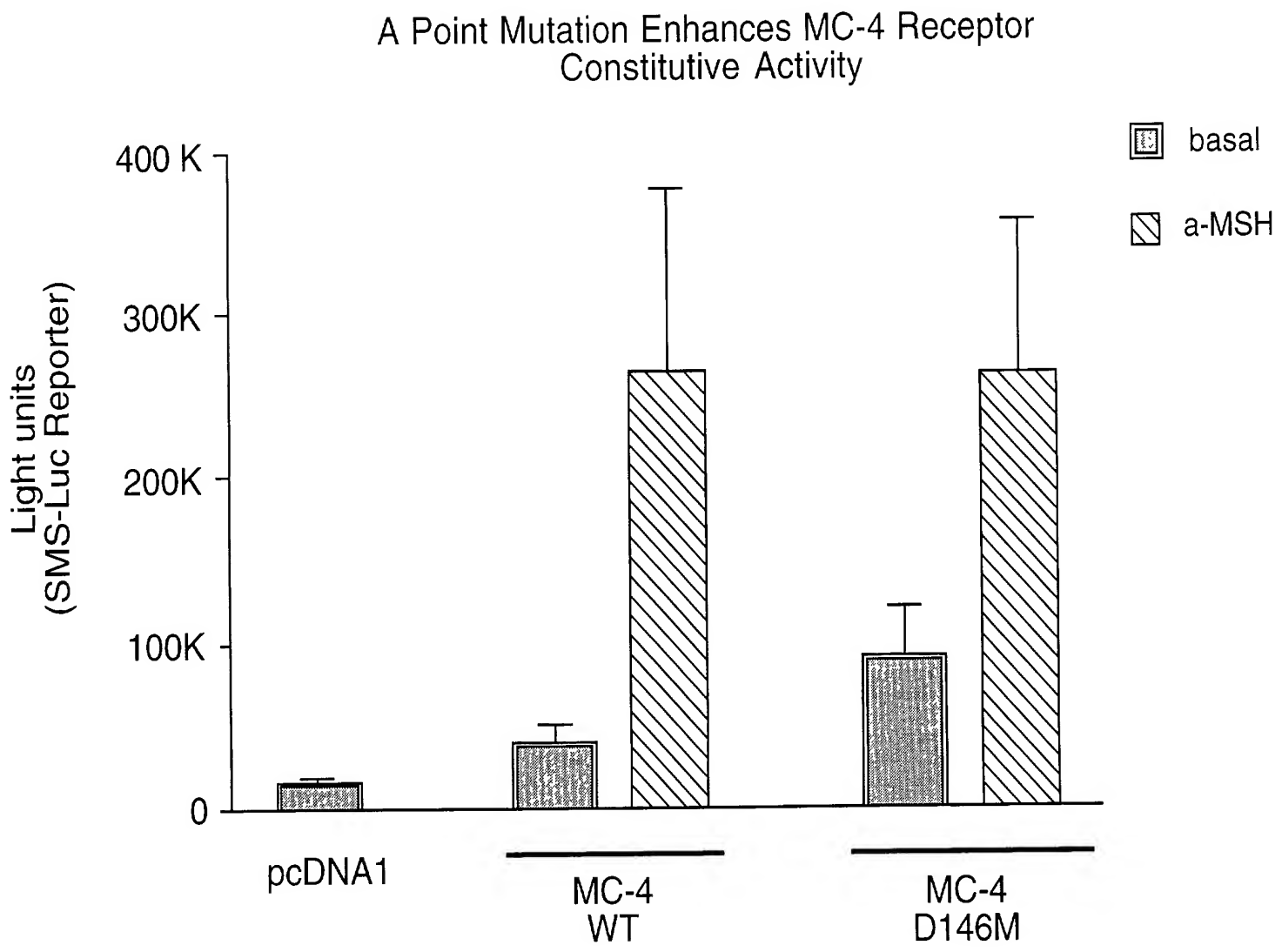
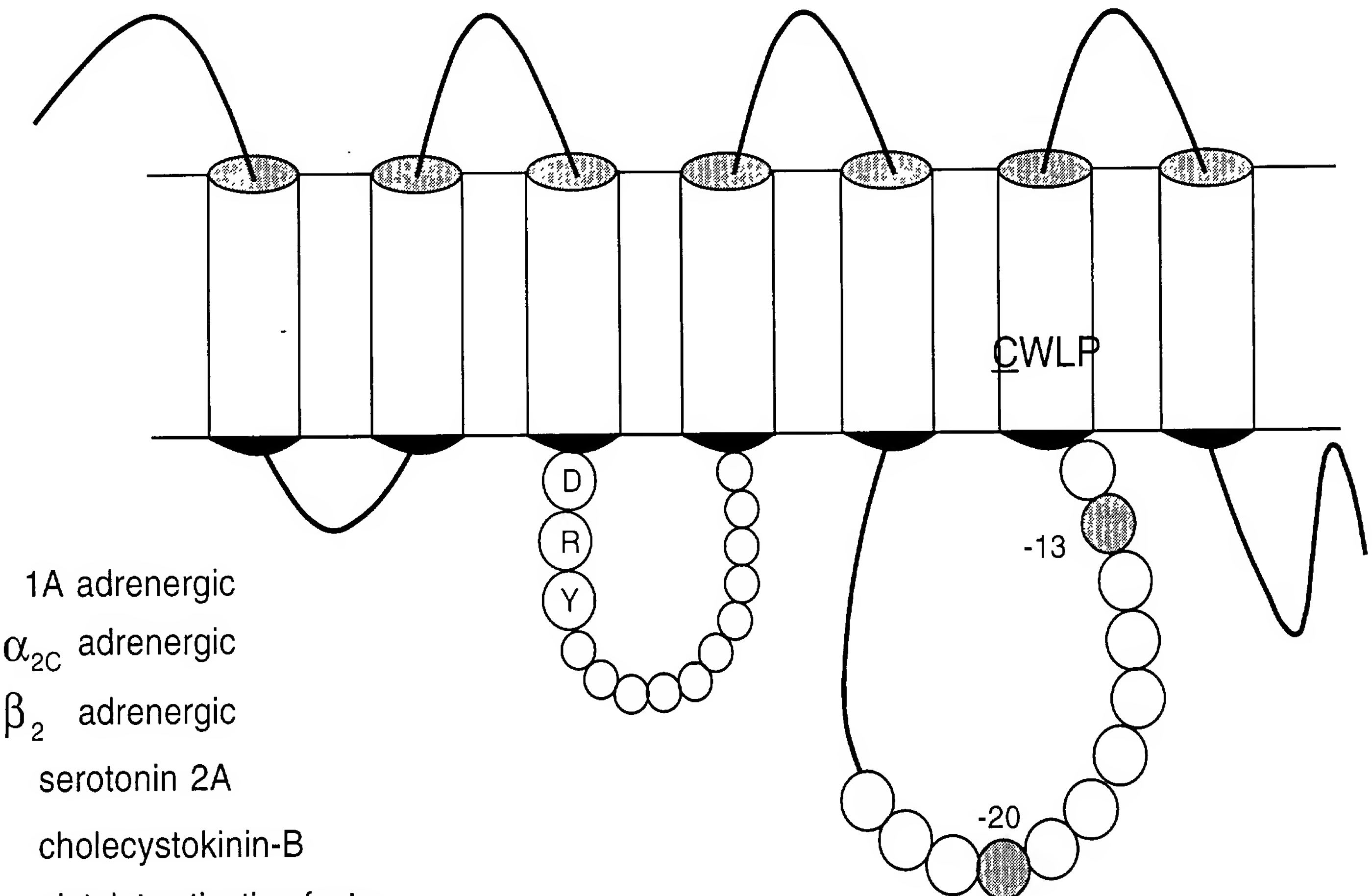

A Point Mutation Enhances MC-4 Receptor Constitutive Activity
basal
a-MSH
Light units (SMS-Luc Reporter)
400 K
300K
200K
100K
0
pcDNA1
MC-4 WT
MC-4 D146M

FIG. 12

The -13 Position is a Target for Mutation Induced Constitutive Activity



1A adrenergic

α_{2C} adrenergic

β_2 adrenergic

serotonin 2A

cholecystokinin-B

platelet activating factor

thyroid stimulating hormone

FIG. 13

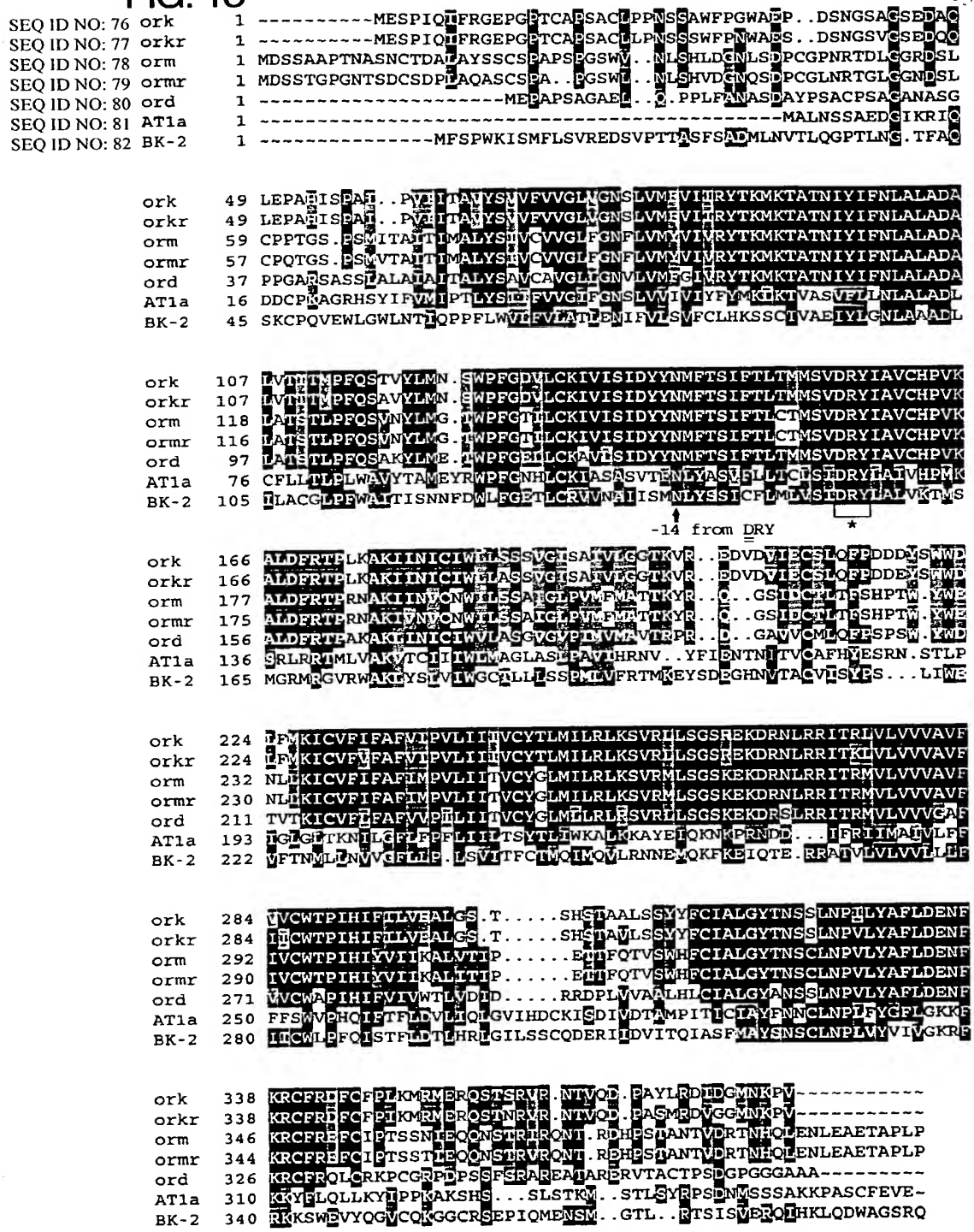

SEQ ID NO: 76 ork
SEQ ID NO: 77 orkr
SEQ ID NO: 78 orm
SEQ ID NO: 79 ormr
SEQ ID NO: 80 ord
SEQ ID NO: 81 AT1a
SEQ ID NO: 82 BK-2
-14 from DRY
*

FIG. 14

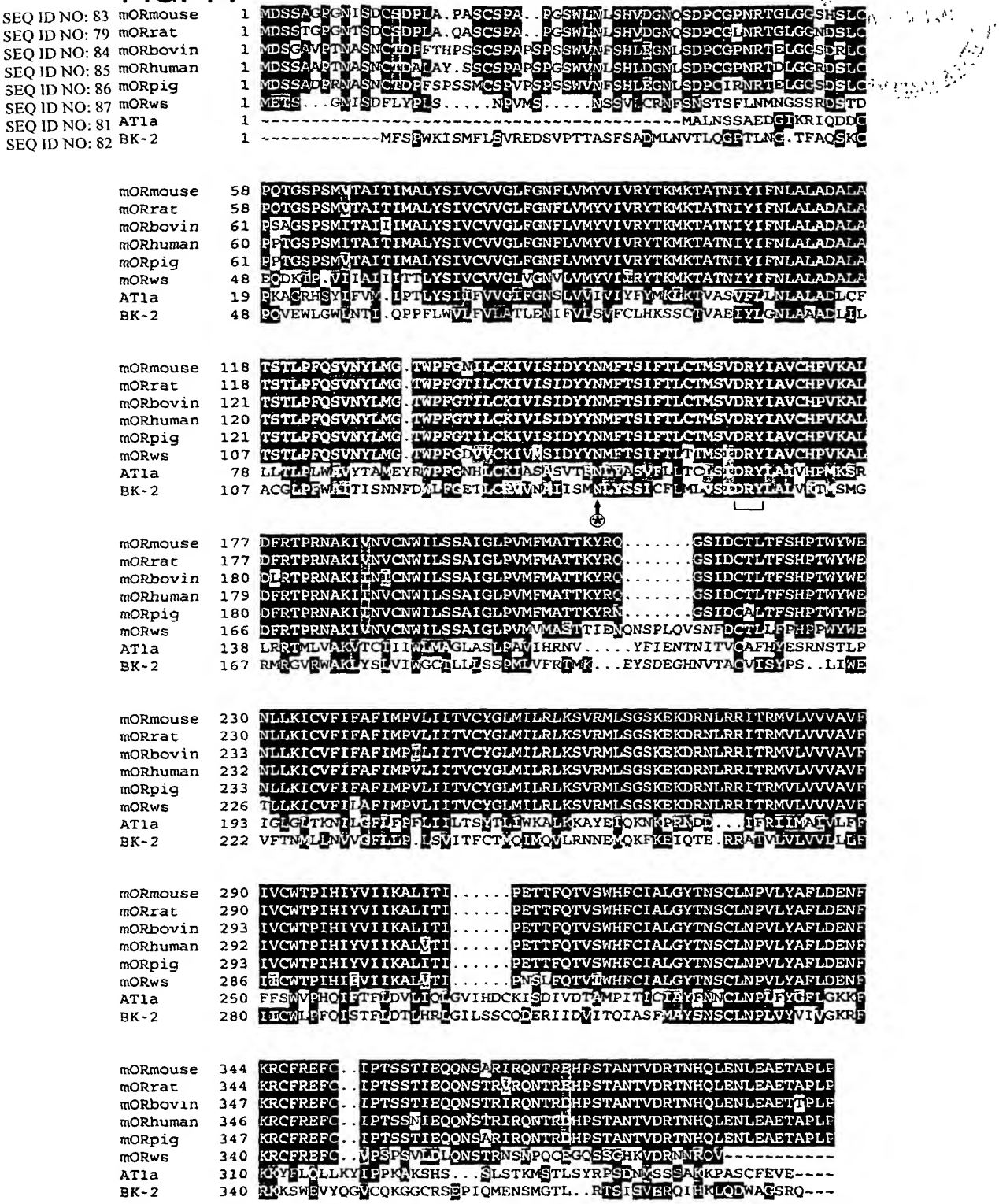

SEQ ID NO: 83 mORmouse
SEQ ID NO: 79 mORrat
SEQ ID NO: 84 mORbovin
SEQ ID NO: 85 mORhuman
SEQ ID NO: 86 mORpig
SEQ ID NO: 87 mORws
SEQ ID NO: 81 ATla
SEQ ID NO: 82 BK-2

FIG. 15

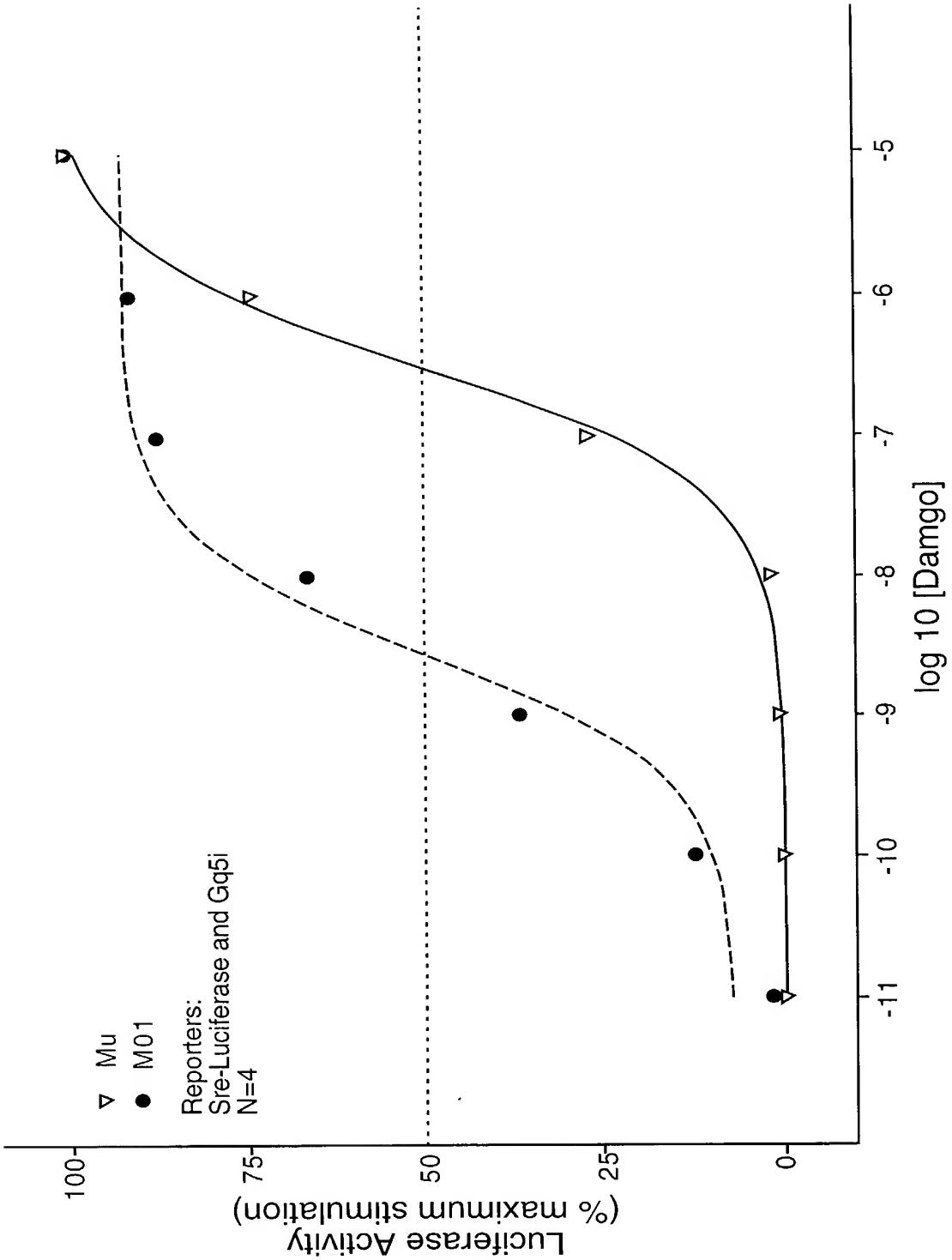

Luciferase Activity
(% maximum stimulation)
100
75
50
25
0
-11
-10
-9
-8
-7
-6
-5
log 10 [Damgo]
Mu
M01
Reporters:
Sre-Luciferase and Gq5i
N=4

FIG. 16

An Intracellular Point Mutation Results in
Loss of Ligand-Induced Function

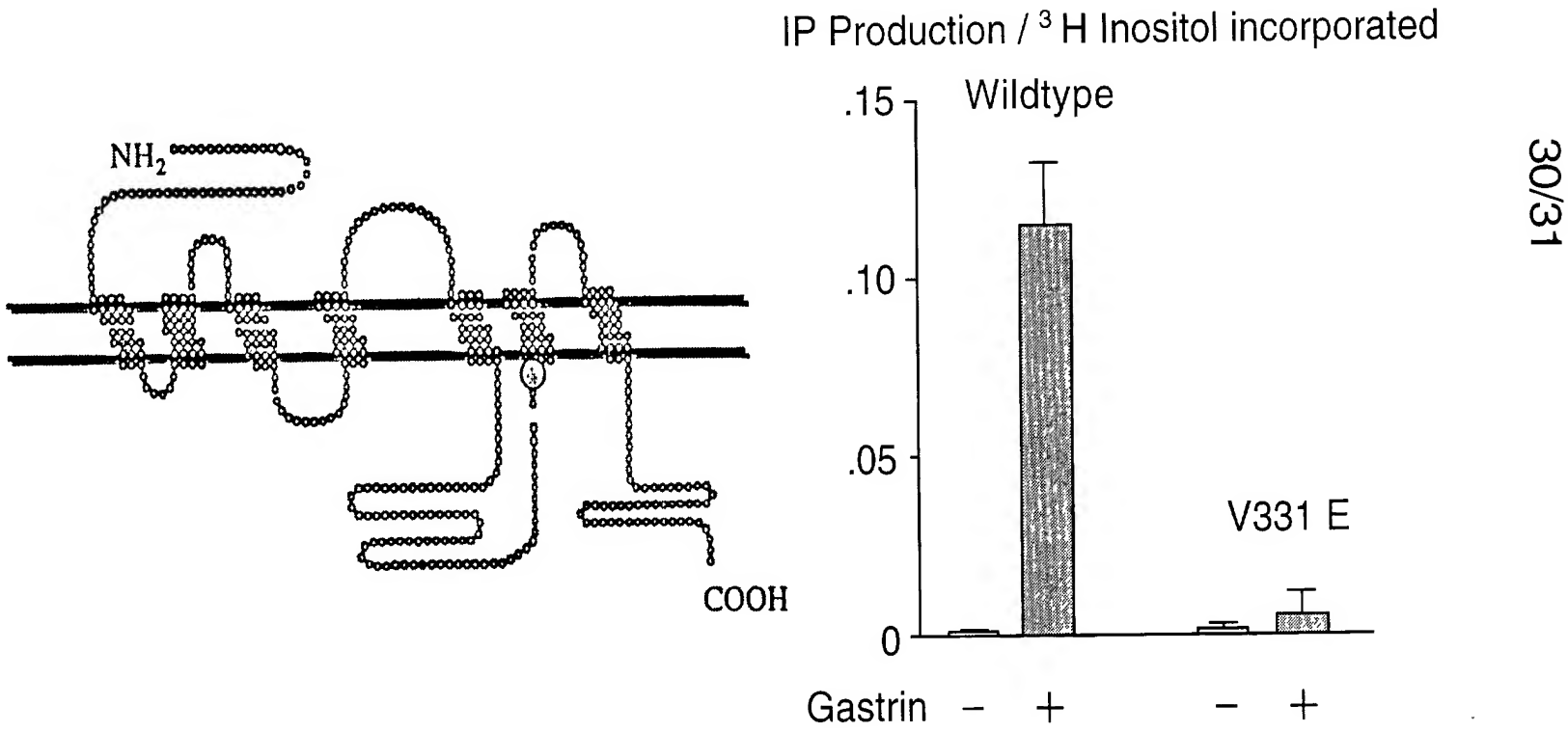
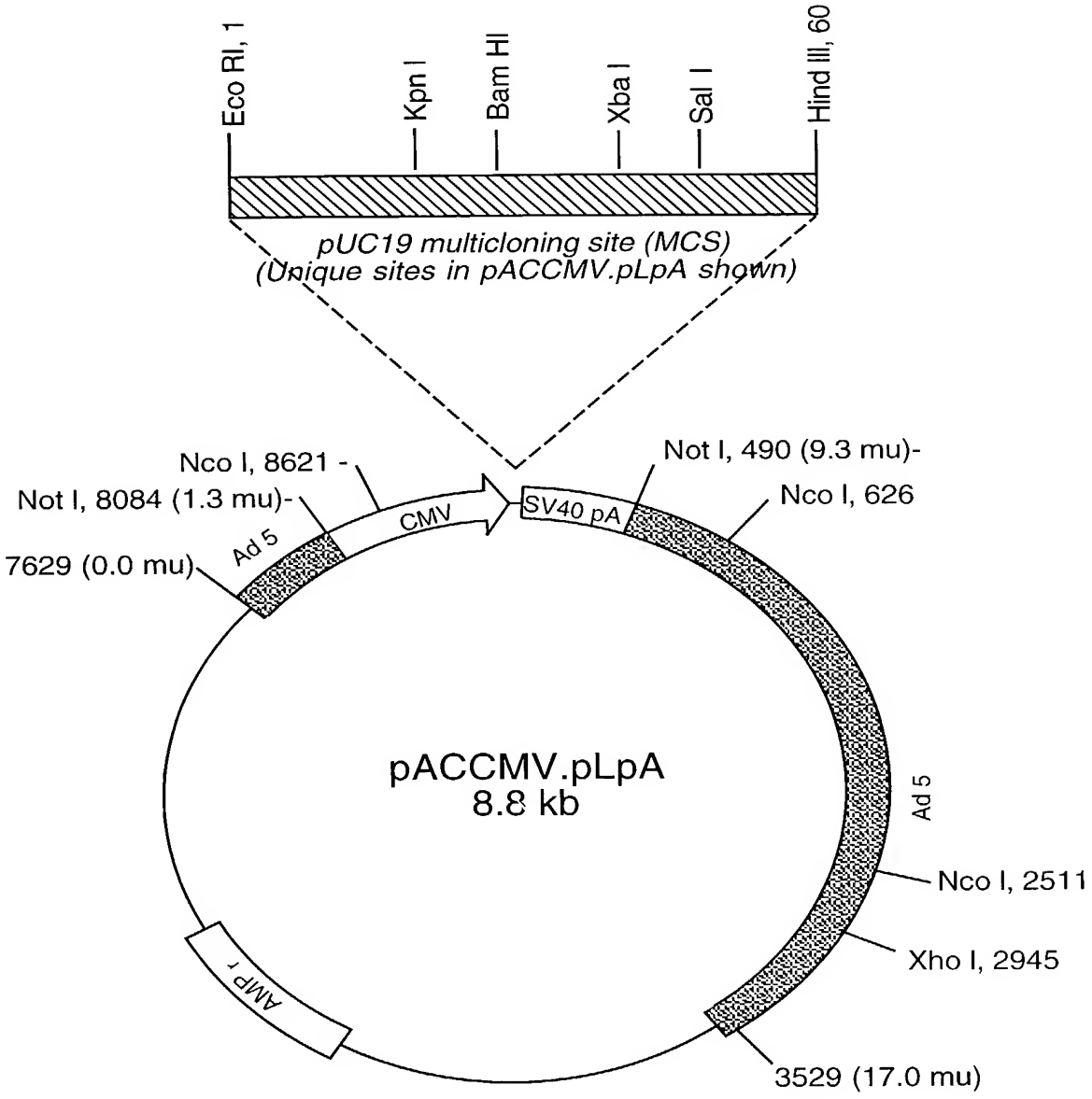


FIG. 17


Eco RI, 1
Kpn I
Bam HI
Xba I
Sal I
Hind III, 60
pUC19 multicloning site (MCS)
(Unique sites in pACCMV.pLpA shown)
Nco I, 8621 -
Not I, 8084 (1.3 mu)-
7629 (0.0 mu)
Ad 5
CMV
SV40 pA
Not I, 490 (9.3 mu)-
Nco I, 626
pACCMV.pLpA
8.8 kb
Ad 5
Nco I, 2511
Xho I, 2945
3529 (17.0 mu)
AMPr